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Biochemical Composition and Bioaccumulation of Heavy Metals in Some Seafood in the Mediterranean Coast of Egypt

Marwa M. Mazrouh* and Mohamed H. Mourad
National Institute of Oceanography & Fisheries, Alexandria-Egypt
*Corresponding author: asmaroza@gmail.com

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ABSTRACT

The main objective of this study is to investigate the variation between biochemical compositions and bioaccumulation of some heavy metals in edible muscle of commercially important seafood, crustacean (*Peneaus japonica*), cuttlefish (*Sepia vulgaris*), mussels (*Donax trunculatus*) and oyster (*Tapes decussates* and *Paphia undulata*) as an attempt to rank them as an alternative rich animal energy source for human and to determine if consumers are at risk of consuming this seafood. The mean values of body constituents differed significantly ($P<0.05$) among various seafood species. The recorded data declare that the highest caloric content was observed in the following order: *Peneaus japonica* > *Paphia undulata* >*Tapes decussates* > *Sepia vulgaris* > *D. trunculatus*. On the other hand, the average concentrations of heavy metals in muscle decreased in the following order: Pb > Cd > As > Hg > Al µg/g wet weight. Moreover, MPI values suggested that *D. trunculatus* and *P. undulata* have a greater capacity for metal bioaccumulation than *P. japonica* and *T. decussates* while *S. vulgaris* had the lowest value. The present study confirmed that the investigate seafood species was safe within the limits for human consumption. So it is recommended that more research and assessment of seafood quality is needed to provide more data and help safeguard the health of human.

INTRODUCTION

Seafood constitutes an important food component for a large section of the world population. Therefore, consumption of seafood has been increased in recent years. The main reason for this increasing demand is that seafoods are very delicious and possess high quality of protein, low cholesterol, a high percentage of n-3 polyunsaturated fatty acids (PUFA), as well as vitamins, essential minerals such as calcium, phosphorus, iron, copper and other nutrient (Zalloua *et al*., 2007; FDA 2009; Okonko *et al*.,2009). Therefore, seafood will be one of the most important sources of animal protein for human consumption in many parts of the world (Speedy, 2003).

Despite their incredible nutrient density, seafood has a reputation for being unhealthy due to their bottom-feeder. The logic goes that bottom-feeders consume the feces, parasites, decomposing animals, and various pollutants that accumulate at the floor of body water.

Among these pollutants, heavy metals which have received more attention from marine scientists during recent years. This is due to their high potential to enter and accumulate in a food chain (Erdoğrul and Erbilir, 2007).

Although seafood species are economically important and widely consumed along Egyptian population, yet little is known regarding their body composition to evaluate and compare caloric values of edible muscles of these marine organisms. Also, there is a gap in information about the prevalence of heavy metals in locally consumed seafood (El Nemr *et al*., 2012; Hamed *et al*., 2013; Shreadah *et al*., 2015). Moreover, some alarm has been expressed concerning health problems associated with seafood consumption due to the presence of certain heavy metals in quantities exceeding those of the maximum permeable limit (MPL) allowed under the standards (El-Serehy *et al*., 2013).

In this context, the present study had three objectives. The first was the determination of the biochemical composition of some Egyptian seafood to use them as an alternative animal protein source. The second was the determination of the residual heavy metal concentrations in the edible muscles. The third was to identify the potential public health risk that could be associated with Egyptian dietary intakes of seafood.

MATERIALS AND METHODS

Samples of seafood (*Peneaus japonica*, *Donax trunculatus*, *Tapes decussates* and *Paphia undulata*) were randomly collected from some local markets at Alexandria Governorate during 2016-2017. About six samples for each organism were placed in small sterile polyethylene plastic bags in an icebox and transferred immediately to the laboratory. Each sample was divided into two parts, the first was used for chemical analysis and the other part kept frozen at -18 ºC for heavy metals analysis. The analysis was carried out on composite samples of 6 specimens of each species having a uniform size.

Determination of body composition

Proximate compositions (protein, fat, carbohydrate) were determined according to the standard analytical procedures of (AOAC, 2002). Caloric values were determined by using conversion factors: 4.20 cal/mg for carbohydrate, 4.19 cal/mg for protein and 9.50 cal/mg for lipid (Prosser and Brown, 1961).

Determination of heavy metals

The contents of Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb) were determined in seafoods muscles samples using an atomic absorption spectrophotometer (AAS) (Perkin Elmer, Waltham, MA, USA, model 1200 A) according to the Standard Method 3110 (APHA, 1992). For each run, three blanks were analyzed using the same procedure in order to check the purity of reagents and any possible contamination.

Metal pollution index

In order to compare the total content of heavy metals at different locations, the metal pollution index (MPI) was calculated according to Usero *et al*., (1997).

$$MPI = (Cf_1 \times Cf_2 \ldots Cfn)^{1/n}$$

Where Cfn = concentration of the metal (n) in the sample expressed in µg/g of wet weight.

Estimated dietary intakes of metals

The human risk assessment has been estimated in this study by comparing the metal intake from consumption rate of seafood with the provisional tolerance weekly intake (PTWI). For the metals Cd, Pb, Hg, As and Al was calculated to be 7, 25, 5, 15 and 1000 µg/kg body weight/week, respectively (Joint FAO/WHO Expert Committee on Food Additives, 2004). Weakly exposure to a given heavy metal was determined according to this formula:

Weakly exposure to a given heavy metal = A concentration of heavy metal in seafood x mean seafood intake/week.

Based on the dietary intake survey by Dawoud, (2005) the local inhabitants had an average consumption per person (70 kg in body weight) of 36.03 g/day for seafood.

Statistical Analysis

Data were recorded as the mean values and the standard deviations (M±SD). Means of six separated seafood were compared using one way ANOVA to determine significant differences where the term significant difference was referred to $p \leq 0.05$. The analysis was carried out using a software program (Graph Pad Prism 5.01 Software).

RESULTS AND DISCUSSION

Proximate chemical composition

Variations of protein, lipid, carbohydrates and energetic values (mean values ± SD) of seafood edible muscle are shown in Table (1). Overall, significant differences ($p < 0.05$) were observed in the chemical composition of seafood. The present study shows that protein contents varied in the ranges of (41.33±3.05 to 68.33±4.32 mg/g). The average value of protein was calculated to be 52.97±2.32 mg/g. Analysis of seafood muscles showed that the highest protein value was achieved in *P. japonica* (68.33±4.32 mg/g) followed by *S. vulgaris* (61.17±3.03 mg/g); *P. undulata* (51.17±2.47 mg/g); and *T. decussates* (42.83±2.09 mg/g). The lowest average protein content was observed in *D. trunculatus* (41.33±3.05 mg/g). These variations in protein contents may be attributed to that the proteins are subject to periodic fluctuations influenced by environmental variables like temperature (Dinakaran *et al*.,2009), or may be due to the physiological, genotype and phenotype differences (Habib *et al*.,2013). On the other hand, lipid contents in the muscle of seafood fluctuated between (20.33±1.69 to 37.33±2.57 mg/g). The average value of lipid was calculated to be 30.60±1.48 mg/g. The highest lipid value was achieved in *P. japonica* (37.33±2.57 mg/g) followed by *P. undulata* (35.33±1.61 mg/g); *T. decussates* (33.83±2.23 mg/g) and *S. vulgaris* (26.17±.2.44 mg/g). The lowest average lipid content was observed in *D. trunculatus* (20.33±1.69 mg/g). It is well known that the lipid content may be influenced by multiple factors, such as feed composition, geographic origin, age variation, reproductive stage and catch season (Murillo, *et al*., 2014). Additionally, carbohydrates contents in the present study were found in the ranges of (11.17±0.95 to 18.83±1.52 mg/g) with an average of 15.30±0.77 mg/g. The highest carbohydrate content was obtained in *T. decussates* (18.83±1.52 mg/g) followed by *P. japonica* (15.67±1.85 mg/g); *S. vulgaris* (15.48±1.89 mg/g) and *P. undulata* (15.33±1.17 mg/g). The lowest value of carbohydrate was found in the *D. trunculatus* (11.17±0.95 mg/g). The low values of carbohydrates recorded in the present study suggest that glycogen in many marine animals does not contribute significantly to the total reserves of the body (Lilly *et al.*, 2017).

On the other hand, whole-body energy content was measured using the sum of energy contributed by total-body lipid, protein, and carbohydrate. The recorded data declared that caloric values in the muscle of seafood fluctuated between (706.80±20.45 to 413.90±26.65 cal./g). The average value of caloric value was calculated to be 578.02±20.35 cal./g. The recorded data confirmed that the highest caloric content was observed in *Peneaus japonica* (706.80±20.45cal./g) followed by *Paphia undulata* (615.20±29.85 cal./g) then *Tapes decussates* (579.10±29.02 cal./g) and *Sepia vulgaris* (575.20±30.18 cal./g) whereas *Donax trunculatus* had less total caloric value (413.90±26.65 cal./g). In this study, it has been clear that there was a positive correlation between total caloric value and lipid content (r= 0.908) as well as a with protein and carbohydrate contents (r= 0.766; 0.632), respectively. These results are in agreement with Shamsan and Ansari, (2010).

Table 1: Biochemical composition of some seafood from the Egyptian coast.

Species	Protein (mg/g)	Lipid (mg/g)	Carbohydrate (mg/g)	Caloric value (cal./g)
P. japonica	68.33±4.32	37.33±2.57	15.67±1.85	706.80±20.45
S. vulgaris	61.17±3.03	26.17±.2.44	15.48±1.89	575.20±30.18
D. trunculatus	41.33±3.05	20.33±1.69	11.17±0.95	413.90±26.65
T. decussates	42.83±2.09	33.83±2.23	18.83±1.52	579.10±29.02
P. undulata	51.17±2.47	35.33±1.61	15.33±1.17	615.20±29.85
Average	52.97±2.32	30.60±1.48	15.30±0.77	578.02±20.35

Data are expressed as means ± S.E. of six separated determinations.
Significant variations (P<0.05) among various seafood species.

Heavy metals accumulation

Heavy metal concentrations (µg/g wet weight) in muscle tissue of some seafood are shown in Table (2). ANOVA data demonstrated significant variations for the most studied metals. It is well known that muscles are not an active site for metal biotransformation and accumulation (Elnabris *et al*., 2013). But in polluted aquatic habitats, the concentration of metals in muscles may exceed the permissible limits for human consumption and imply severe health threats. Looking to the residual heavy metals in edible muscles of seafoods organisms in the present work, it was clear that the mean order of metals accumulation in seafood was as follow, Pb (1.190) >Cd (1.054) >As (1.041) > Hg (0.706) > Al (0.700) µg/g wet weight. The range of heavy metals in samples of seafood was 0.882-1.187 µg/ g wet sample for As, 0.468-0.923 µg/ g wet sample for Hg, 0.968-1.545 µg/ g wet sample for Pb, 0.915-1.178 µg/ g wet sample for Cd, and 0.528-0.873 for Al.

In this study, the higher levels of As were found in *D. trunculatus* (1.187±0.08), followed by *P. undulata* (1.132±0.07), *T. decussates* (1.005±0.10), *P. japonica* (0.998±0.13) while the lowest contents were detected in *S. vulgaris* (0.882±0.11) µg/ g wet sample. No significant (P>0.05) difference was a record between the five species. As is the most toxic element and considered as a Group A human carcinogenic and can effects mainly to the lung, kidney, and skin disorder (ATSDR, 2003). It is well known that sea foods concentrate arsenic in seawater, but it exists in the organic forms, which have not been shown to produce adverse effects in humans consuming this seafood. This type of organic arsenic is also rapidly excreted (FDA, 2009). The mean As concentration (1.041±0.0.05 µg/g) in the present study was considerably lower than the maximum level (3.5 µg/ g wet weight) set by Canadian guideline (2014). Moreover, the maximum As level reported by Georgian Food Safety Rules (2001) for fish is 2.0 µg/ g wet weight.

Table 2: Bioaccumulation of heavy metals (µg/g wet weight) in some seafood.

Species	As	Hg	Pb	Cd	Al
P. japonica	0.998±0.13	0.923±0.06	0.968±0.80	1.100±0.08	0.583±0.08
S. vulgaris	0.882±0.11	0.898±0.06	0.995±0.10	0.915±0.10	0.528±0.06
D. trunculatus	1.187±0.08	0.570±0.06	1.545±0.17	1.093±0.08	0.873±0.07
T. decussates	1.005±0.10	0.468±0.05	1.413±0.13	0.983±0.06	0.773±0.14
P. undulata	1.132±0.07	0.667±0.09	1.027±0.11	1.178±0.06	0.742±0.03
Average	1.041±0.05	0.706±0.04	1.190±0.08	1.054±0.04	0.700±0.04

Average of 6 observation± standard error.
Significant variations (P<0.05) among various seafood species
Non Significant variations for As and Cd (P>0.05).

Regarding to Hg, the present data show that *P. japonica* accumulated the highest content of Hg (0.923±0.06) in their edible muscles followed by *S. vulgaris* (0.898±0.06), followed by *P. undulata* (0.667±0.09) and *D. trunculatus* (0.570±0.06) while the lowest levels were observed in *T. decussates* (0.468±0.05) µg/ g wet sample. A significant (P<0.05) difference was a record between the five species. The obtained results are in agreement with Ahdy *et al*., (2007) who found that the highest levels of Hg were recorded in the prawn. Mercury is one of the most toxic elements among the studied heavy metals and exposure to high level of this element could permanently damage the brain, kidneys and developing fetus (Castro-González and Méndez-Armenta, 2008). The mean Hg concentration (0.706±0.04 µg/g) in the present study was considerably higher than the maximum level (0.50 µg/ g wet weight) set by the Commission Regulation (EC, 2006).

Furthermore,the present results detected that *D.trunculatus* species accumulated higher content of Pb (1.545±0.17) followed by *T.decussates* (1.413±0.13) species followed by *P.undulata* (1.027±0.11) and *S.vulgaris* (0.995±0.10). While the lowest contents were detected in *P. japonica* (00.968±0.80) µg/g wet sample. A significant (P<0.05) difference was recorded between the five species. Lead is known to damage the cardiovascular system, kidneys, liver and the reproductive system (Assi, 2016). The mean Pb concentration in the present study (1.190±0.08 µg/g) was considerably lower than the maximum level (2.0, 1.5 ug/g wet weight) set by FAO/WHO, (2004).

Additionally, higher levels of Cd were found in *P. undulata* (1.187±0.08) followed by *P. japonica* (1.100±0.08), *D. trunculatus* (1.093±0.08), *T. decussates* (0.983±0.06). The lowest contents of Cd were detected in *S. vulgaris* (0.915±0.10) µg/ g wet sample. This result is in agreement with Abdel- Salam and Hamdi, (2014). No significant (P>0.05) difference was recorded between the five species. Cadmium has been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic (Lippmann, 2000; Risso-de-faverney *et al*., 2001). Cadmium levels recorded in this study were above the acceptable limits for human consumption for crustacean (0.50 µg/g) and for bivalve and cephalopods (1.0 µg/g) as recommended by CEC (2006).

Finally, the present data show that the contents of Al had the following decreasing order: *D. trunculatus* (0.873±0.07) > *T. decussates* (0.773±0.14) > *P. undulata* (0.742±0.03) > *P. japonica* (0.583±0.08) > *S. vulgaris* (0.528±0.06) µg/g wet sample. A significant (P<0.05) difference was recorded between the five species. Aluminum is a harmful metal to the aquatic ecosystem, being responsible for events of toxicity with serious ecological consequences (Correia, *et al*., 2010). Different physiological alterations frequently observed in different fish species exposed to Al as cardiovascular, hematology, respiratory, ion regulatory, reproductive, metabolic, endocrine and gill damage (Brodeur, *et al*., 2001; Barcarolli and Martinez, 2004; Vuorinen, *et al*., 2003).

Generally, the concentrations of heavy metals in aquatic organisms depend on feeding habits (Canli and Atli, 2003; Uysal *et al*., 2009), size, age (Rashed, 2001, Fernandes *et al*., 2007), lifestyle and exposure durations to contaminants of different species (Canli and Kalay, 1998) and the rate of metal detoxification (Urena *et al*., 2007). Moreover, many laboratory and field studies reported that metal accumulation in tissues of organisms depends on metal concentration in the water and also the exposure period, Physico-chemical parameters such as the temperature, pH, salinity, and hardness of the water play a crucial role in heavy metal accumulation (Clearwater *et al*., 2002; Tuzen, 2003; Yılmaz *et al*., 2012). Finally, it was observed that the levels of heavy metals in seafood are often considerably higher than in other constituents of marine environment due to their habitat and their feeding habits (Canli and Atli, 2003, Sun *et al*., 2011). Compared to sediments, seafood also exhibits greater spatial sensitivity and therefore, is the most reliable tool for identifying sources of biologically available heavy metal contamination (El-Sikaily *et al*., 2004; Hamed and Emara, 2006).

Metal Pollution Index

To compare the total metal content in the different seafood species investigated in this study, the metal pollution index (MPI) was determined. As shown in Table (3), MPI values suggested that *D. trunculatus* (0.985±0.045) and *P. undulata* (0.918±0.047) have a greater capacity for metal bioaccumulation than *P. japonica* (0.880±0.051) and *T. decussates* (0.849±0.033), while, *S. vulgaris* (0.795±0.024) had the lowest value. A significant ($P<0.05$) difference was recorded between the five species. Abdel-Salam and Hamdi, (2014) reported that sepia samples had the lowest MPI between commercially important crustaceans and mollusks collected from Egyptian and Saudi Arabia coasts. Moreover, metal pollution index for the bivalve, crustacean, and cephalopods collected from Alexandria region ranged from 0.074 to 0.490 (Ahdy *et al*., 2007).Therefore, it is suggested that *D.trunculatus* and *P. undulata* are more vulnerable to metal pollution than the other studied species and can be used as a bioindicator of metal pollution.

Table 3: Metal pollution index (MPI) in different seafood.

Species	MPI
P. japonica	0.880±0.051
S. vulgaris	0.795±0.024
D. trunculatus	0.985±0.045
T. decussates	0.849±0.033
P. undulata	0.918±0.047
Average	0.885±0.021

Average of 6 observation± standard error.

Human risk assessment

The human risk assessment is well known to estimate by comparing the metal intake from consumption rate of seafood with the provisional tolerance weekly intake (PTWI). To our knowledge, there have been few reports on the assessment of potential risks of heavy metals in seafood collected in Egyptian coast to date. As shown in Table, (4) the seafood intake of 252.21g per week for those adults who consume seafood gives an exposure to metals that in all cases falls well within the JECFA safe intake levels. So, we can say that, although heavy metal levels in Egyptian seafood exceed the maximum permissible limit in some samples, only the consumption of seafood species from Egyptian coast is safe on human health and do not pose any health risks.

Table 4: Estimated dietary exposure of the seafood-consuming adult population to metals from Mediterranean Egyptian coast.

Metal	Mean dietary exposure to metal (ug/week)	PTWI* for a reference adult (ug/week)
As	262.55	1050
Hg	178.06	350
Pb	300.13	1750
Cd	165.82	490
Al	176.55	7000

* Provisional tolerance weekly intake.

CONCLUSION

From this study we conclude that the intake of metals depends not only on the levels of metals in seafood but also on the amount consumed. The obtained results are in agreement with El-Nemr *et al*., (2012). Thus many governments have provided dietary advice to consumers to limit consumption where levels of pollution are elevated. The data obtained help to assess the state of heavy metals pollution of several marine species, which is a necessary step in environmental protection and to understand the role of biota in the accumulation of heavy metals from water, which is a part of bio-mechanism in aquatic ecosystems. Finally, it is recommended that more research and assessment of seafood quality is needed to provide more data and help safeguard the health of human.

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ARABIC SUMMARY

التركيب البيوكيميائى والتراكم البيولوجى للمعادن الثقيلة فى بعض المأكولات البحرية فى ساحل البحر المتوسط

مروة محمود مزروع ـ محمد حسن مراد
معمل فسيولوجيا الاسماك ـ شعبة المصايد ـ المعهد القومى لعلوم البحار والمصايد- الاسكندرية

الهدف الرئيسي من هذه الدراسة هو دراسة التباين بين التركيبات الكيميائية الحيوية والتراكم الحيوي لبعض المعادن الثقيلة في العضلات الصالحة للأكل للمأكولات البحرية ذات الأهمية التجارية ، مثال: القشريات (*Peneaus japonica*) ، والحبار (*Sepia vulgaris*) ، وام الخلول (*Donax trunculatus*) والمحار (*Tapes decussates*)و(*Paphia undulata*) محاولة لترتيبها كمصدر بديل للطاقة الحيوانية الغنية للإنسان وتحديد ما إذا كان المستهلكون عرضة لخطر استهلاك هذه المأكولات البحرية. اختلفت القيم المتوسطة لمكونات الجسم بشكل كبير ($P < 0.05$) بين الأنواع المختلفة من المأكولات البحرية. واظهرت النتائج أن أعلى محتوى من السعرات الحرارية لوحظ بالترتيب التالي:-

Peneaus japonica > *Paphia undulata* >*Sepia vulgaris* > *D. trunculatus*.

من ناحية أخرى،انخفض متوسط تركيزات المعادن الثقيلة في العضلات بالترتيب التالي:- Pb> Cd> As> Hg> Al وعلاوة على ذلك ، اظهرت قيم MPI أن يكون لكل من *D. trunculatus* و *P. undulata* قدرة أكبر على التراكم للمعادن من *P. japonica* و *T. decussates* بينما كانت قيمة *S. vulgaris* أقل قيمة.

أكدت الدراسة الحالية أن الأنواع البحرية التي تم التحقيق فيها آمنة في حدود الاستهلاك البشري.

نوصى بضرورة المزيد من البحث والتقييم لجودة المأكولات البحرية لتوفير المزيد من البيانات للحفاظ على صحة الإنسان.